SUPPLEMENTAL MATERIAL

Carotid Body Denervation Improves Autonomic and Cardiac Function and Attenuates Disordered Breathing Patterns in Congestive Heart Failure

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SUPPLEMENTAL METHODS

Evaluation of Heart Rate Variability

Heart rate was derived from dP/dt blood pressure signal recorded at 10 kHz sampling rate from the radiotelemetry transmitter (Data Science International, USA). Heart rate was calculated on a beat-to-beat basis by using the Powerlab system and LabChart software (ADInstruments) and heart rate variability (HRV) was analyzed by using the HRV extension for LabChart 7 as previously described(Pliquett *et al*, 2003). Briefly, beat-to-beat intervals were transformed by a fast Fourier transformation algorithm followed by Hann windowing at 256 points with 50% overlap. HRV in the time domain was analyzed for the SD of interpulse intervals (SDNN). Power spectral analysis in the frequency domain was performed using the following frequency cutoffs: 0.0625–0.1875 Hz were considered as the low-frequency (LF) band, and 0.1875–0.5625 Hz as the high-frequency (HF) band(Moguilevski *et al*, 1995). Total power integrated the whole spectrum.

Evaluation of Systolic Blood Pressure Variability (LFSBPV)

Changes in the low frequency (LF) component of systolic blood pressure variability (LF SBPV) reflect the level of sympathetic vasoconstrictor activity(Stauss, 2007). The same BP data used to calculate HRV were used for SBPV analysis in the same analytical manner as for HRV analysis (Egi et al, 2007). SBP was derived from the blood pressure signal recorded at 10 kHz sampling rate from the radiotelemetry transmitter (Data Science International, USA). A fast Fourier transformation algorithm followed by Hann windowing at 256 points with 50% overlap was applied to the SBP.

Power spectral analysis in the LF domain (0.0625-0.1875 Hz) was performed to estimate LFSBPV.

Spontaneous cardiac baroreflex sensitivity (cBRS)

Spontaneous cBRS was assessed by fast Fourier transform (FFT) of the R-R interval and the systolic blood pressure (SBP) in the low frequency domain since the coupling between the R-R and SBP in the high frequencies are not of baroreflex origin(Parati *et al*, 2000). R-R interval and SBP was obtained on a beat-to-beat basis using radiotelemetry (DSI, USA). Data was transformed using an FFT algorithm following by Hann windowing at 256 points with 50% overlap. Spectral calculation of the baroreflex gain (α-coefficient) was calculated as the squared root of the ratio of R-R interval and SBP powers in the LF domain (LF: 0.0625–0.1875 Hz). The α-coefficient was calculated at 3 different time points each 5 minutes in length.

Spontaneous renal sympathetic baroreflex sensitivity (rBRS)

Spontaneous rBRS was determined by relating spontaneous changes in renal sympathetic nerve activity (RSNA) and diastolic blood pressure (DBP)(Sundlof and Wallin, 1978). Briefly, DBP (measured by radiotelemetry) and RSNA recordings were obtained when the animal was at rest in the plethysmograph. Data were grouped in 1 mmHg DBP bins during 5 min baseline recordings. Within these bins, RSNA burst incidence was determined and plotted against the corresponding DBP. Spontaneous rBRS was defined as the slope between RSNA burst incidence and DBP bins after linear regression analysis. Slopes with an r-value greater than 0.4 were used for analysis (Sundlof and Wallin, 1978).

Table S1. Resting mean arterial pressure and heart rate (day 3 post CB surgery)

sham-sham	pre-pace		Post CB surgery (day 3)
MAP (mmHg)	67 ± 2		73 ± 8
HR (beats/min)	201 ± 14		201 ± 10
CHF-sham	pre-pace	CHF	Post CB surgery (day 3)
MAP(mmHg)	64 ± 7	63 ± 5	57 ± 6 #
HR (beats/min)	212 ± 10	258 ± 29	225 ± 15
CHF-CBD	pre-pace	CHF	Post CB surgery (day 3)
MAP (mmHg)	73 ± 6	64 ± 5	71 ± 2†
HR (beats/min)	215 ± 6	233 ± 14	217 ± 5

MAP = mean arterial pressure. HR=heart rate. n = 6 sham-sham, n = 7 CHF-sham, n = 8 CHF-CBD. # $p < 0.05$ compared to sham-sham at respective time point, † $p<0.05$ compared to CHF-sham at respective time point.

Table S2. Resting mean arterial pressure and heart rate (day 6-9 post CB surgery)

sham-sham	pre-pace		post-surgery (day 3)	post-surgery (day 6-9)
MAP (mmHg)	67 ± 2		76 ± 8	68 ± 8
HR (beats/min)	195 ± 14		194 ± 8	189 ± 12
CHF-sham	pre-pace	CHF	post-surgery (day 3)	post-surgery (day 6-9)
MAP(mmHg)	68 ± 4	63 ± 5	62 ± 2#	64 ± 7
HR (beats/min)	211 ± 12	254 ± 35	227 ± 14	218 ± 13
CHF-CBD	pre-pace	CHF	post-surgery (day 3)	post-surgery (day 6-9)
MAP (mmHg)	84 ± 8#	67 ± 4*	70 ± 3*†	80 ± 5
HR (beats/min)	217 ± 10	253 ± 25	215 ± 5	219 ± 13

MAP = mean arterial pressure. HR=heart rate. n = 5 in all groups. * $p<0.05$ compared to pre-pace, # $p< 0.05$ compared to sham-sham at respective time point, † $p<0.05$ compared to CHF-sham at respective time point.

Table S3. Resting Breathing (day 3 post CB surgery)

sham-sham	pre-pace		post-surgery (day 3)
RR (breaths/min)	241 ± 45		220 ± 41
V_t (mL)	1.2 ± 0.2		1.4 ± 0.2*
V_e (mL/min)	276± 61		277 ± 45

CHF-sham	pre-pace	CHF	post-surgery (day 3)
RR (breaths/min)	189 ± 45	239 ± 60	166 ± 31
V_t (mL)	1.2 ± 0.1	1.5 ± 0.3	1.6 ± 0.2
V_e (mL/min)	218 ± 41	288 ± 30	246 ± 38

CHF-CBD	pre-pace	CHF	post-surgery (day 3)
RR (breaths/min)	278 ± 36	247 ± 30	123 ± 24*†
V_t (mL)	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.1*
V_e (mL/min)	288 ± 24	310 ± 38	150 ± 17*†‡

RR = respiratory rate. V_t = tidal volume. V_e = minute ventilation. n = 6 sham-sham, n = CHF-sham, n = 8 CHF-CBD * $p < 0.05$ compared to pre-pace, † $p < 0.05$ compared to CHF, ‡ $p < 0.05$ compared to sham-sham at respective time point.

Table S4. Resting Breathing (day 6-9 post CB surgery)

sham-sham	pre-pace		post-surgery (day 3)	post-surgery (day 6-9)
RR (breaths/min)	216 ± 45		180 ± 58	168 ± 45
V_t (mL)	1.2 ± 0.2		1.7 ± 0.2*	1.7 ± 0.2*
V_e (mL/min)	236± 56		267 ± 64	262 ± 46
CHF-sham	pre-pace	CHF	post-surgery (day 3)	post-surgery (day 6-9)
RR (breaths/min)	193 ± 37	252 ± 29	143 ± 30†	142 ± 46†
V_t (mL)	1.2 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	1.4 ± 0.2
V_e (mL/min)	224 ± 34	251 ± 30	191 ± 46	194 ± 63
CHF-CBD	pre-pace	CHF	post-surgery (day 3)	post-surgery (day 6-9)
RR (breaths/min)	245 ± 37	164 ± 20*‡	84 ± 13*†	59 ± 6 *†‡
V_t (mL)	1.2 ± 0.1	1.4 ± 0.1‡	1.6 ± 0.1	1.9 ± 0.2*
V_e (mL/min)	272 ± 27	224 ± 23	133 ± 27*†	110 ± 13*†

RR = respiratory rate. V_t = tidal volume. V_e = minute ventilation. n=5 in all groups. * $p< 0.05$ compared to pre-pace, † $p< 0.05$ compared to CHF, ‡ $p<0.05$ compared to CHF-sham at respective time point.

Table S5. Heart Rate and Blood Pressure Variability Analysis (day 3 post CB surgery)

sham-sham	pre-pace		post-surgery (day 3)
SDNN HRV	15.0±1.2		13.5±1.2
Total Power HRV (ms^2)	203.7±25.3		178.4±36.9
LF HRV (ms^2)	25.2±4.1		30.2±3.4
HF HRV (ms^2)	44.8±4.4		37.5±3.8
LF/HF HRV ratio	0.6±0.2		0.6±0.1
cBRS	1.0±0.1		1.2±0.1
LF SBPV (%)	100±0.0		90.2±15.1

CHF-sham	pre-pace	CHF	post-surgery (day 3)
SDNN HRV	15.2±1.9	8.7±1.4*	10.9±1.7
Total Power HRV(ms^2)	245.2±56.1	100.0±37.4*	126.6±28.5*
LF HRV (ms^2)	26.0±3.3	31.5±5.5	27.3±8.0
HF HRV (ms^2)	43.5±6.0	24.7±3.2*	28.5±4.3*†
LF/HF HRV ratio	0.7±0.2	1.4±0.3*	1.0±0.3
cBRS	1.2±0.1	0.9±0.1*	0.9±0.1*†
LF SBPV (%)	100±0.0	234.2±52.3*	212.2±55.0*†

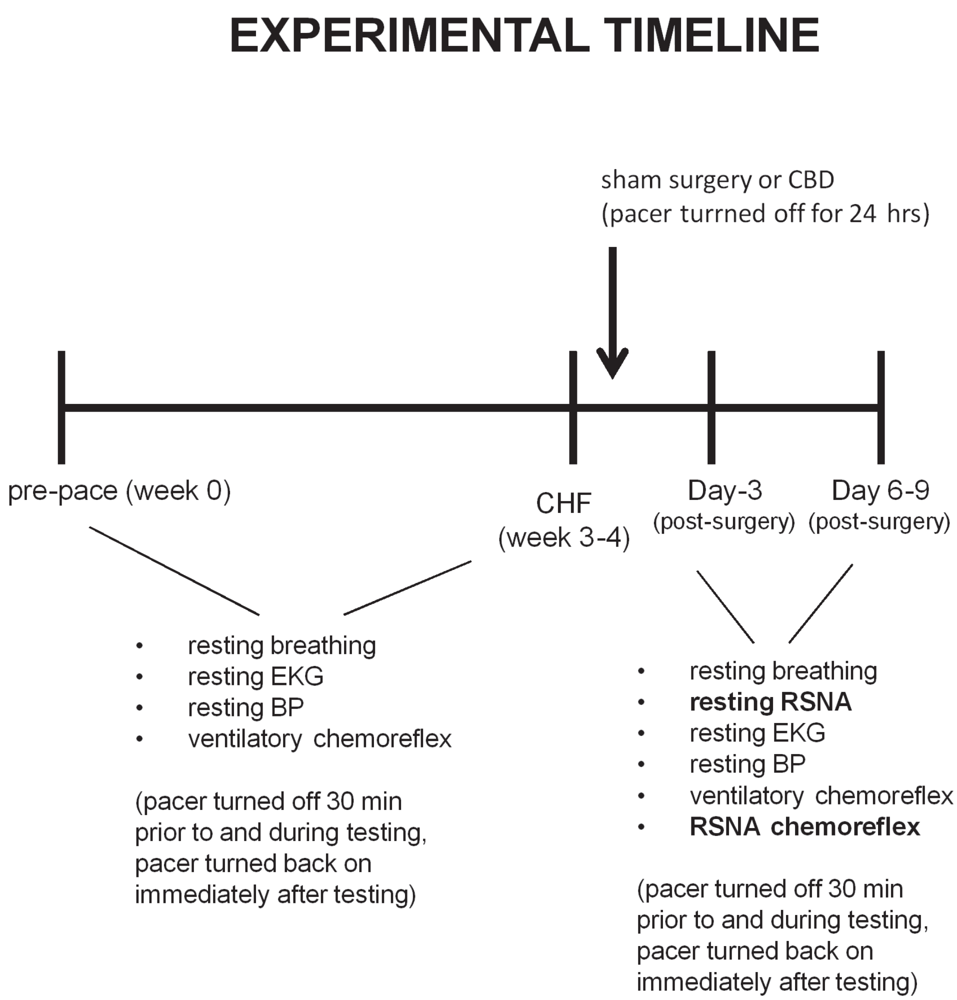
SDNN = standard deviation of the normalized RR interval; LF = low frequency power; HF = high frequency power; LF SBPV = low frequency power of systolic blood pressure variability. cBRS, cardiac baroreflex sensitivity. See Fig. S1 for group n. * $p < 0.05$ compared to pre-pace, † $p < 0.05$ compared to sham-sham (day 3).

Table S6. Heart Rate and Blood Pressure Variability Analysis (day 6-9 post CB surgery)

sham-sham	pre-pace		post-surgery (day 3)	post-surgery (day 6-9)
SDNN HRV	14.4±1.7		14.4±1.5	14.8±2.5
Total Power HRV (ms^2)	198.3±37.6		202.8±51.4	157.3±28.1
LF HRV (ms^2)	22.0±3.1		25.9±2.7	23.5±4.6
HF HRV (ms^2)	42.7±2.7		33.4±2.2	40.8±4.5
LF/HF HRV ratio	0.5±0.1		0.8±0.1	0.6±0.1
cBRS	1.0±0.1		1.2±0.8	1.1±0.1
LF SBPV (%)	100.0±0.0		91.5±23.1	95.8±22.4

CHF-sham	pre-pace	CHF	post-surgery (day 3)	post-surgery (day 6-9)
SDNN HRV	17.6±2.0	10.4±2.6*	13.1±2.7	10.3±1.4*
Total Power HRV (ms^2)	189.2±76.5	50.6±30.1	107.7±38.3	83.9±20.9
LF HRV (ms^2)	23.9±5.1	30.7±9.8	32.3±9.9	18.2±6.2
HF HRV (ms^2)	49.5±9.9	31.0±5.5*	35.0±5.8*	19.3±7.9*†‡
LF/HF HRV ratio	0.6±0.3	1.0±0.2	1.1±0.5	1.2±0.3
cBRS	1.5±0.5	1.0±0.1	1.0±0.0	1.0±0.1
LF SBPV (%)	100.0±0.0	275.9±150.4	253.5±135.0	338.1±177.6

SDNN = standard deviation of the normalized RR interval; LF = low frequency power; HF = high frequency power; LF SBPV = low frequency power of systolic blood pressure variability. cBRS, cardiac baroreflex sensitivity. See Fig. S1 for group n. * $p < 0.05$ compared to pre-pace, † $p<0.05$ compared to CHF, ‡ $p < 0.05$ compared to 3 day post-neck surgery time point.



sham-sham, n=6 to Day 3 post CB surgery, of which n=5 had viable RSNA signal at Day 6-9

CHF-sham, n=7 to Day 3 post CB surgery, of which n=5 had viable RSNA signal at Day 6-9

CHF-CBD, n=8 to Day 3 post CB surgery, of which n=5 had viable RSNA signal at Day 6-9

Figure S1. Experimental Timeline and Experimental Groups.

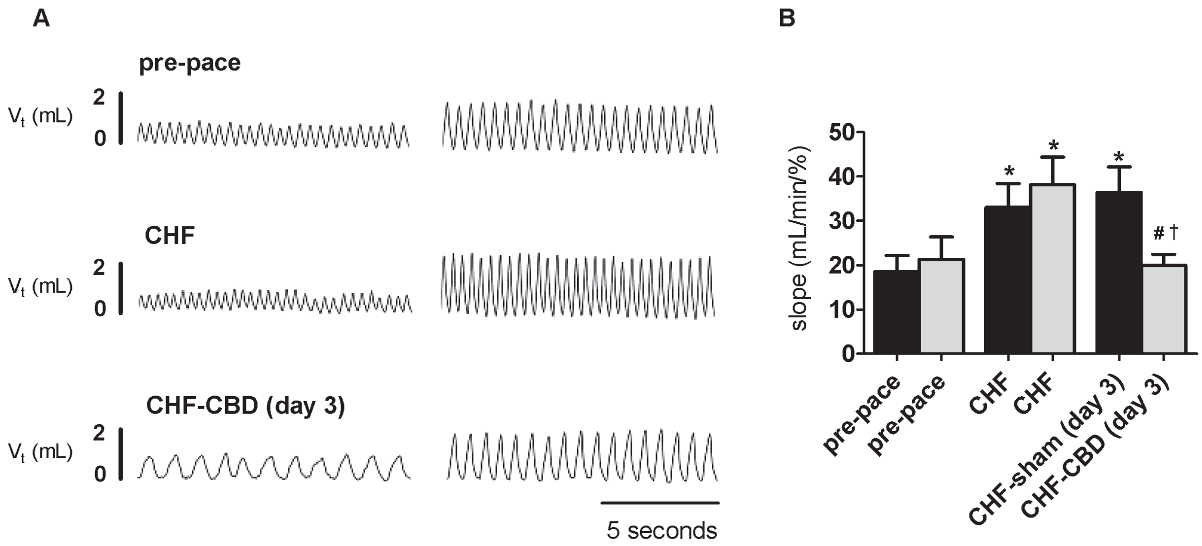


Figure S2. Effect of CHF and CBD on Ventilatory Response to CO_2

Representative tidal volume (V_t) tracings (**A**) and summary data (**B**) illustrating that the slope (sensitivity) of the minute ventilatory (V_E) response to CO_2 inhalation (5% and 7% CO_2 in normoxic gas) was enhanced in CHF, and reduced after CBD. Ventilatory responses to CO_2 were not altered in sham-sham group (data not shown) and were the same as prepace values in the CHF groups. n= 7 CHF-sham, n = 8 CHF-CBD. * p<0.05 vs. pre-pace. # p<0.05 vs. CHF-sham. † p<0.05 vs. CHF time point in the same group before CB surgery.

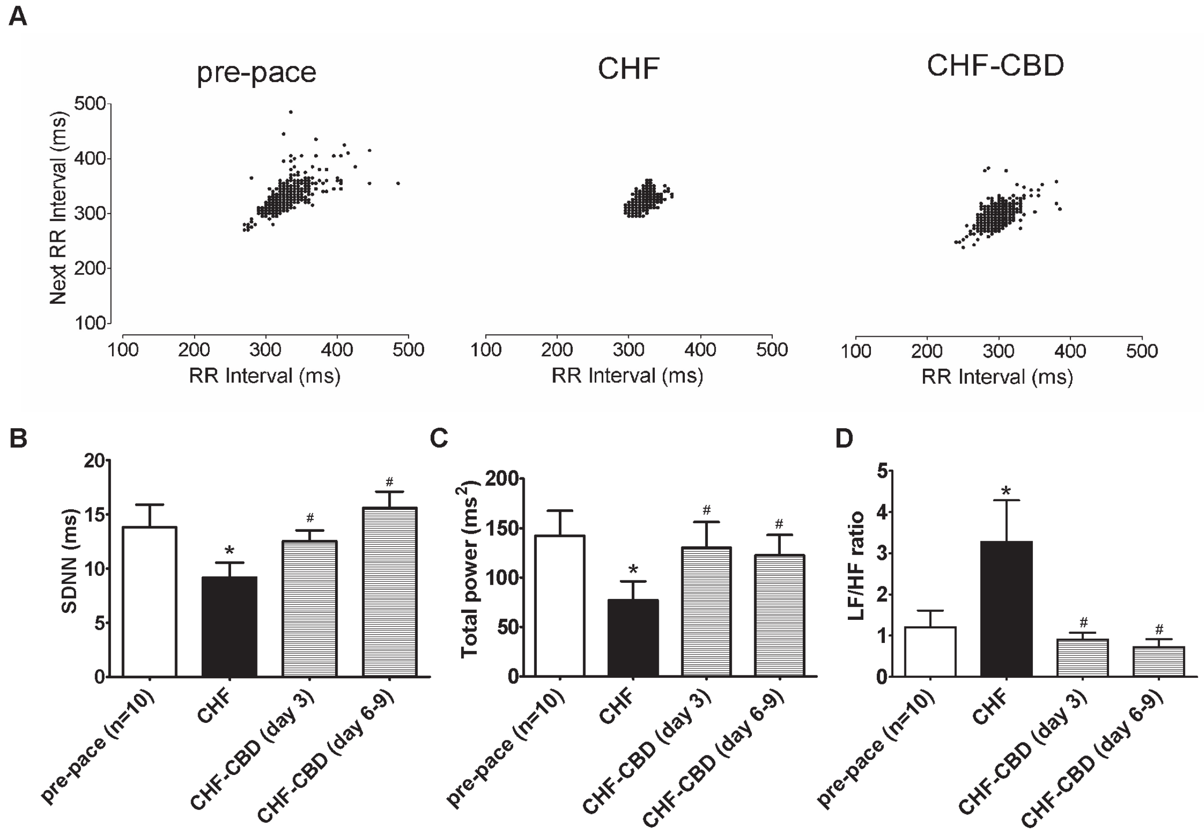


Figure S3. Effect of CBD on Heart Rate Variability in CHF.

(A) Poincare plots illustrating heart rate variability (HRV) in a rabbit in the pre-pace, CHF, and CHF-CBD states. **(B & C)** HRV analysis in the time (B) and frequency (C) domains. The standard deviation of the normal r-r interval (SDNN) and the total spectral power were reduced in CHF and partially normalized after CBD. (**D**) The low frequency to high frequency ratio (LF/HF) of HRV was increased in CHF, and CBD normalized this effect. Data are longitudinal comparisons within the CHF-CBD group through the course of the study. Data for sham-sham and CHF-sham groups are shown in Tables S5 and S6. *$p < 0.05$ vs. pre-pace, $^{\#}p < 0.05$ vs. CHF

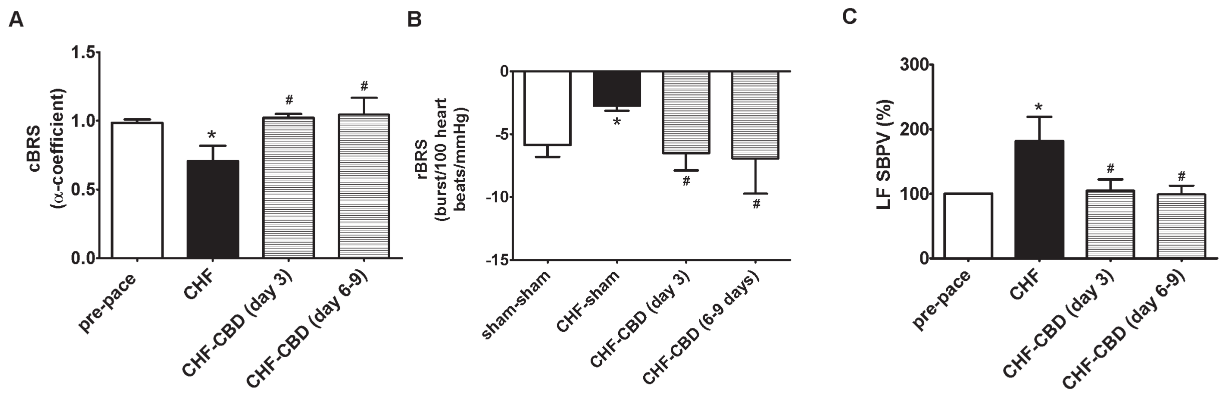


Figure S4. Effect of CHF and CBD on Cardiac Baroreflex and Renal Sympathetic Baroreflex Function and Sympathetic Vasomotor Tone in CHF.

Both cardiac (cBRS) (α-coefficient) **(A)** and renal sympathetic **(B)** baroreflex sensitivity were decreased in CHF and restored with CBD. **(C)** The low frequency component of systolic blood pressure variability (LV-SBPV) was increased in CHF and was reduced after CBD. **A** and **C** are longitudinal comparisons within the CHF-CBD group through the course of the study. Data for cBRS and LF-SBPV from sham-sham and CHF-sham groups are shown in Tables S5 and S6. See Fig. S1 for group n. *$p < 0.05$ vs. pre-pace, $^{\#}p < 0.05$ vs. CHF.

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